

## DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney Docket No. 3339-239A

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

### METHOD FOR THE REGULATION OF PROTEIN BIOSYNTHESIS

the specification of which	
is attached hereto	
OR	
was filed on as United States Application No. or PCT International Application Number	er
and was amended on (if applicable).	
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.	
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.	ì
I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or	

§ 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

92 06765	France	June 4, 1992	Yes No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			Yes No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			Yes No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date (MM/DD/YYYY)
Application Number	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application (37 C.F.R. § 1.63(d)).

PCT/FR 92/00524	June 2, 1993	National Phase Entered
Application No.	Filing Date	Status Patented/Pending/Abandoned
08/347,353	December 1, 1994	Pending
Application. No.	Filing Date	Status Patented/Pending/Abandoned
Application No.	Filing Date	Status Patented/Pending/Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

## **Customer Number 000826**

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Full name of (first/sole) inventor:

Joel Sternheimer

Inventor's

Signature: Joil Hercheimen Jim Date: 5/19/

Residence:

Citizenship:

French

Post Office Address:

CLT01/4358048v1

ADDRESS: \_\_\_

☐ Individual

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney's Docket No. 3339-239A

Nonprofit Organization

Applicant, Patentee, or Identifier: Application No. or Patent No.: Filed or Issued: Title:	Joel Sternheimer To Be Assigned Concurrently Herewith METHOD FOR THE REGULATION OF PROTEIN BIOSYNTHESIS				
	CLAIMING SMALL ENTITY STATUS and 1.27(b))INDEPENDENT INVENTOR				
As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office described in:					
the specification filed the application identified the patent identified a					
law to assign, grant, convey, or licer qualify as an independent inventor u	yed, or licensed, and am under no obligation under contract or use, any rights in the invention to any person who would not under 37 C.F.R. § 1.9(c) if that person had made the would not qualify as a small business concern under 37 ration under 37 C.F.R. § 1.9(e).				
Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:					
	cern, or organization exists. oncern, or organization is listed below.				
FIII NAME:					

Small Business

FULL NAME:					
ADDRESS: Individual Small Business Nonprofit Organization					
Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 C.F.R. § 1.27)					
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b))					
Joel Sternheimer NAME OF INVENTOR					
Tiel Skribeiner TSM (Signature of Inventor)					
Date 19/ 17/95					
CLT01/4358044v1					

#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:

Joël Sternheimer

Appl. No.:

To Be Assigned

Filed: For:

Concurrently Herewith METHOD FOR THE REGULATION OF

PROTEIN BIOSYNTHESIS

May 25, 1999

Assistant Commissioner for Patents Washington, DC 20231

## RULE 132 DECLARATION OF JOËL STERNHEIMER

Sir:

- I, JOËL STERNHEIMER, do hereby declare and say as follows:
- That I am a graduate of Paris, Lyons and Princeton Universities and received my degrees in the years 1964, 1966 and 1967 (Doctorate in 1966).
   My Curriculum Vitae and the list of publications are attached thereto (Annexes 1 and 2).
- 2. That it clearly emerges from the Invention as now defined that it does not lack utility: the method of the invention allows and controls the *in situ* regulation of the synthesis of selected protein. It was undertaken by Mr. Pedro Ferrandiz under my supervision to stimulate the growth of blue-green algae—prokaryotes, genus Anabaena- by epigenetic regulation. Their photosynthetic activity involves in particular pigmentary proteins (cyanins). Thus their biosynthesis is easily observed through color change and oxygen release.

We want to point out that this first attempt of stimulation in an aquatic medium is relatively simple to reproduce. We believe that the results obtained are particularly promising. One may add the fact that it points towards numerous applications.

#### • Materials and methods

- Dilution of 12 ml of Anabaena variabilis (stock provided by the Ecole Normale Supérieure de Paris) in 1 500 ml of mineral water.
- Addition of 40 g of dry vegetable manure containing 8%, say 2.6 g, of nitrates as well as 40 g of river pebbles (as suggested by Vincent Bargoin this would provide the solution with trace elements).
- Adaptation time to the cultures medium: four days.
- Transfer of 750 ml of the solution in two vats subjected to natural enlightment.

  This setting in culture started on the 30<sup>th</sup> of April.

Musical diffusions.

The music has been diffused in one of the vats, by mean of an aquatic speaker Altec UW-30, while the other vat served as a control.

The proteins transcripted in musical sequences were the following:

- TAPE I (45 min)

NIF H of Anabaena v. (five times)

Allophycocyanin of Anabaena v. (three times)

Plastocyanin of Anabeana v. (three times)

Nitrate reductase of Chorella s. (three times)

PS1 photosystem protein of Anabaena v. (three times) (\*)

Ferredoxin of Anabaena v. (five times)

Protein 35 K of Anabaena v. (eight times) (\*).

- TAPE II (15 min)

Allophycocyanin of Anabaena v. (two times)

Plastocyanin of Anabaena v. (two times)

PS1 photosystem protein of Anabaena v. (three times) (\*)

Ferredoxin of Anabaena v. (four times)

Protein 35 K of Anabaena v. (eight times).

- TAPE III (15 min)

Ferredoxin of Anabeana v. (two times)

NIF H of Anabeana v. (three times)

NIF A of Anabeana v. (three times) (\*)

NIF D of Anabeana v. (three times) (\*)

Nitrate reductase of Chlorella s. (three times)

Protein 35 K of Anabeana v. (two times) (\*).

The transcriptions had been realized by J. Sternheimer on a sampler Casio SK1 apart from those labelled (\*) which were made by P. Ferrandiz on a « One Key Play » software written by Sylvie Guillou and Fabrice Ocelli (INSERM St-Anne, Paris). The rate of the transpositions is tuned so as to make their length correspond to the photoperiods of the micro-organisms.

Tape I was played twice a day, from the 30<sup>th</sup> of April to the 5<sup>th</sup> of May. Then from the 7<sup>th</sup> to the 10<sup>th</sup> of May TAPE II was played in the morning while TAPE III was played in the evening.

During this period the viability of the micro-organisms was regularly controlled: Samples were drawn from the cultures and then checked under a microscope.

#### • Results (Annex 3)

- Evolution of the coloration of cultures (Figure 1)

One poured in the vats the solutions looked opaque (after tossing).

This was due to the manure mentionned above, the dilution rate of the original stock but also to the spread of a fibrous contaminant which was not characterized.

From the 2<sup>nd</sup> day of listening the musical vat presented a greater proportion of suspending matter than the control one. However this trend reversed itself by the 4<sup>th</sup> day. We therefore assumed that the musical exposures had been too long and we decided to abort the diffusion of Tape I.

Instead Tapes II and III have been used. We then observed on May 8<sup>th</sup> that the tint of the cultures in the musical vat displayed a green blue coloration more pronounced than those in the control vat (Figure 2).

This trend kept increasing up to the end of the experiment.

- Oxygen release.

Ten days after the end of the period of diffusion the musical cultures became characterized by a proliferation of bubbles at the surface (Figure 3).

Since these bubbles had the property to revive the flame of a lighted match which was put close by, we concluded they contained oxygen. On May 24<sup>th</sup> there were about 70 surface bubbles and on the 28<sup>th</sup> they were 130 (Figure 4). We point out that the maximum number of visible bubbles observed in the control vat is 8. Hence there is more than a factor 16 between the two cultures with respect to oxygen release. In fact the medium of the musical culture was saturated with oxygen at the end of the observation time. Clearly this is correlated to an increase of the photosynthetic activity in the musical vat. It indicates that while the oxygen was released some carbonated composites have been fixed (Figure 5, taken six months later). Thus this particular application of the epigenetic regulation process led to an interesting depollutive system. This should beget further interests.

Other experiences showing the utility of the instant invention are herewith attached (Annexes 4-8).

As regards a garden experience: See Annex 9.

Figure 1 of this Annex 9 also attached is a comparative test:

On the left side (control): non treated tomatoes

On the right side: tomatoes having received during 16 days 3 minutes per day, the music of protein of anti-drought protein TAS14. Both control tomatoes and the treated tomatoes having 1 1½ litres of water per plant per day.

3. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

4. Further declarant saith not.

Joal Sternhamen

Joël STERNHEIMER

1999/5/. 19

Date